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Leading articles

Postantibiotic effect and host-bacteria interactions

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Suppression of bacterial growth that persists after short exposure of bacteria to antimicrobial agents, called the postantibiotic effect (PAE), has been observed both *in vitro* and *in vivo*. Duration, and even presence or absence of PAE depends upon the organism, the antimicrobial agent and the environmental conditions used for measuring the effect (Craig, 1991). The most important factors contributing to PAE include concentration, exposure time and mode of action of the tested drug, type of organism, inoculum size, and type of growth medium, and growth phase (see Craig & Gudmundsson, 1991). Despite the recognition of PAE many years ago by Bigger (1944), and then by Eagle, Fleischman & Musselman (1950), the mechanism by which it occurs remains to be elucidated. Possible mechanisms include non-lethal damage induced by the antimicrobial agent and drug persistence at the binding site (Craig & Gudmundsson, 1991). PAEs are multiple, including delayed recovery of proteins with and without enzyme activities, prolonged changes in cell morphology, metabolic alteration, modifications of generation times, susceptibility to phagocytosis and altered susceptibility to antibiotics after re-exposure. Many of these effects are probably linked (Mackenzie & Gould, 1993).

Altered susceptibility to polymorphonuclear (PMN) cells during the PAE phase is known as the post antibiotic leucocyte effect (PALE) (McDonald, Pruul & Wetherall, Bayer & Tu, 1990; Pruul & McDonald, 1990; Bayer *et al.*, 1991). PALE was found to be both strain- and drug-dependent. In *Escherichia coli* aminoglycosides and macrolides (protein synthesis inhibitors) displayed greater PALE than the cell-wall acting β-lactam antibiotics (McDonald *et al.*, 1983). Erythromycin increased the susceptibility of *Streptococcus pneumoniae* to the bactericidal activity of human PMNs but decreased that of *E. coli*. Exposure to roxithromycin increased opsonization and phagocytosis in *S. pneumoniae* but not in *Streptococcus pyogenes* (Gemmell & McLeod, 1992). Erythromycin and to a lesser extent azithromycin increased the susceptibility of streptococci to the killing activity of human PMNs not only during the PAE phase but also after recovery (Ramadan *et al.*, 1994). Erythromycin and roxithromycin but not azithromycin altered opsonization and increased the susceptibility of *Staphylococcus aureus* to phagocytosis by human PMNs (Pascual *et al.*, 1990). A short exposure to vancomycin increased the susceptibility of *Enterococcus faecalis* to PMNs (Bayer & Tu, 1990). The combination of ampicillan/sulbactam enhanced the suscepbility of *Staphylococcus aureus* to phagocytosis by PMNs, in contrast, and probably due to low intracellular penetration of β-lactams, the same combination displayed no significant antimicrobial effect against the bacterium when it was intracellularly located, within the PMNs (Pascual *et al.*, 1991). Short exposure of *Pseudomonas aeruginosa* to supra-MIC concentrations of amikacin increased killing by PMNs even in absence of opsonization (Bayer *et al.*, 1991).

One possible explanation for the changes in bacteria-PMN interactions would be that the antibiotic induces modifications to the cell surface which enhance bacterial susceptibility to phagocytosis or intracellular killing. Supporting this theory are the morphological changes observed in bacteria in relation to PAE. Lorian, Ernst & Amaral (1989), using phase contrast microscopy to follow the bacterial morphology of *E. coli* after exposure to ampicillin and ciprofloxacin observed rapid filamentation of bacteria. Transmission electron microscopy showed that, during PAE, dicloxacillin increased the number of cross-walls and rifampicin produced cell wall thickening in *S. aureus* while intracellular electron dense aggregates were seen in *P. aeruginosa* after exposure to imipenem

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(Gottfredsson *et al.*, 1993). Morphological alterations, notably enlargement, were observed in *S. aureus* exposed to macrolides for 2 h (Watanable *et al.*, 1992). β-Lactam antibiotics with high affinity for PBP2 (such as imipenem) cause a prolonged PAE on Gram-negative bacteria as well as spheroplast prolactin (Gudmundsson, Vogelman & Craig, 1986). In *E. coli* filamentous shapes occur after treatment with enoxacin and ciprofloxacin for 1 h; spheroplasts were observed after treatment with imipenem for 2 h (Hanberger *et al.*, 1993).

Other studies suggest that the PAE may have an impact on toxin and bacterial enzyme production, which, in turn may affect the host. Guan & Burnham (1992) investigated extra- and intracellular-haemolysin activity in *E. coli* during PAE phase. Following exposure to and removal of quinolone, the extracellular haemolytic activity of *E. coli* decreased significantly for at least 2 h, whereas intracellular activity was adversely affected for only 1 h. Thus, the production of haemolysin, but not its export from the bacterium was thought to be affected during the PAE phase. We have demonstrated that roxithromycin inhibits haemolysin production by *S. pyogenes* during the PAE phase and beyond (Shibl, Ramadan & Tawfik, 1994).

Adherence of treated bacteria is also altered during the PAE phase. Bacterial adherence is influenced by the net surface charge and/or specific binding arrangement by host factors and by strain variation. During the PAE phase, the residual antibiotic may cause leakage of bacterial adhesins 1 (β-lactams) or may suppress the formation and expression of adhesins (aminoglycosides) (Lorian, 1991). *E. coli* treated with subinhibitory concentrations of ampicillin attach less well than untreated control bacteria in contrast to those exposed to chloramphenicol or nitrofurantoin (Sandberg, Stenqvist & Svanborg-Eden, 1979). We have also found that cell surface charge (hydrophobic/hydrophilic) of streptococci to hydrocarbon is altered after exposure to macrolides (Ramadan *et al.*, 1994; Shibl *et al.*, 1994). The decrease in hydrophobicity was unrelated to inhibition of growth and may be explained on the basis that macrolides inhibit adhesin synthesis in streptococci (Shibl, 1985).

Further work is needed to clarify the possible impact of PAE in the clinical situation. The concept of PAE should not only be considered as prolonged suppression of bacterial growth but also as a potential inducer of decreased microbial virulence which may directly or indirectly influence the host-parasite relationship.

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Evolution of the bacterial dihydrofolate reductase inhibitors

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Sir,

Numerous pyrimidine and purine analogues were synthesized in the late 1940s as potential nucleic acid antagonists. Particular interest was devoted to antagonists of thymine. It was quickly realized that the 2,4-diaminopytimidines inhibit folic acid utilization and that this was a general property of all of these compounds (Hitchings *et al.*, 1950; Hitchings, 1989).

It was recognised that the 3,4,5-trimethoxyl-diaminopyrimidine-derivative was outstanding for its breadth of antibacterial activity. Consequently it was selected for detailed study and clinical trials in 1959 (Roth *et al.*, 1962) and given the name trimethoprim. Subsequently a 3,4-dimethoxy-5-bromo derivative was also found to be active. The role of bromine substitution on the benzyl-ring of trimethoprim was later confirmed with an analogue in which the 4-methoxy group, instead of being in the 5 position, was substituted by this halogen; the compound was synthesized in 1972 (Kompis *et al.*, 1980) and named brodimoprim. The effect of this substitution was to improve the trimethoprim binding to bacterial DHFR (Then & Hermann, 1984), providing equivalent or better antibacterial activity, improving lipid solubility and the pharmacokinetic behaviour, and to achieve sufficiently high concentrations at the infection site (Weidekamm, 1993).

Trimethoprim was put forward as a potentiator of sulphonamides for the treatment of bacterial infections: sulphamethoxazole was chosen as the sulphonamide owing matched half-life of trimethoprim. It was argued that the combination of sulphamethoxazole and trimethoprim, later given the name co-trimoxazole in Europe, offered several antimicrobial advantages (Hitchings, 1989).

Since its introduction in 1968 co-trimoxazole is still widely used for the treatment of a wide variety of bacterial infections, toxoplasmosis and in particular *Pneumocystis carinii* pneumonia. However, in many circumstances, it is the trimethoprim moiety of this combination that is responsible for its clinical effectiveness owing to increasing bacterial resistance to the sulphonamide. In fact, the sulphonamide moiety of the mixture may be disadvantageous because of a number of adverse reactions and drug interactions which are more common in the elderly and chronically ill patients, or in those receiving prolonged high dose treatment (Reeves, 1982; Kucers, Bennett & Kemp, 1987; British National Formulary, 1993; Brumfitt & Hamilton-Miller, 1994). Trimethoprim became available for use as a single drug in 1973 and brodimoprim 20 years later (Periti *et al.*, 1993).

Trimethroprim alone has been shown in clinical trials to be as effective and better